THE EFFECTS OF HYDROSTATIC PRESSURE UPON THE POLAR LOBE AND CLEAVAGE PATTERN IN THE CHAETOPTERUS EGG

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It was first shown by Marsland (1936) that a hydrostatic pressure of 400 atmospheres "reversed" the incompleted cleavage furrow of a dividing *Arbacia* egg. If the pressure was then released within a short period of time no furrow again appeared until time had elapsed for the normal formation of the second cleavage. At that time both the first and second cleavage furrows cut in almost simultaneously. Marsland (1936, 1938 and 1939*a*) interpreted the reversal of the incutting cleavage furrow as due to the liquefaction of the cortical gel. Pease and Marsland (1939) have confirmed this effect with eleven other eggs. In addition, cortical solation has been demonstrated in *Amoeba* (Marsland and Brown, 1936), in the tentacles of suctorian Protozoa (Kitching and Pease, 1939), and Marsland (1939*b*) has shown plasmagel solation in *Elodea*.

This apparently general effect of hydrostatic pressure upon cortical gels seemed applicable to a study of the formation and properties of the "polar lobe" apparatus present in some spirally cleaving eggs. The egg of *Chaetopterus pergamentaceus* Cuv. proved admirable material. The early cleavage has been described by Lillie (1906). The polar lobe, although relatively small, prominently appears just prior to the first and second cleavages. A small lobe appears before the third cleavage. The lobe persists until just after the cleavage furrows are completed. The first cleavage is very unequal, the second produces two equal *A* and *B* blastomeres, the slightly larger *C* cell, and the much larger *D* blastomere which contains most of the original lobe material. The third cleavage is equatorial and is very nearly equal. The inequality of the first two cleavages accentuates any irregularities in the cleavage pattern which result from pressure. The differential distribution of various granules, in addition to the position of the polar lobe and polar bodies, serve as markers of the normal egg axes and allowed an accurate determination of the cell origins in the 2-, 4-, and 8-cell stages irrespective of the cell size.

The method, in general, was to apply 270–470 atmospheres pressure to the eggs during the formation of the polar lobe, and before the cleavage furrow had cut entirely through the egg. The pressure apparatus has been briefly described by Pease and Kitching (1939), and was so constructed that material could be kept under constant microscopical observation while under pressure. Control eggs, kept in the bomb without pressure, showed no deleterious effects. Controls were also kept for each experiment and when these had completed cleavage, 3½–8 minutes after the pressure was applied to the experimental material, the pressure was released. The eggs so treated were fixed in the 2-, 4-, and 8-cell stages, and, in addition, series of photomicrographs were made at intervals while the eggs were in the bomb to supplement the preserved material.

Results

It was found that a pressure of 220 atmospheres sufficed to suppress the formation of the polar lobe and block cleavage if the pressure was applied during the early stages of lobe formation and before the cleavage furrow had cut deeply. Higher pressures were necessary to reverse deeply cut furrows, and a pressure of 270 atmospheres caused the fully formed polar lobe and all but the deepest cut furrows to be withdrawn and the cell to round up.

After removal of the pressure, when the control eggs had completed cleavage, the eggs remained round until the time for the normal second cleavage to begin, plus a delay approximately equal to the time they were held under pressure. Then the polar lobe re-formed and the first cleavage furrow appeared. Most frequently the second cleavage furrow appeared almost simultaneously and the egg divided at once into four cells with the cleavage planes along the polar axis (Figs. 6–8, egg *A*). The third cleavage appeared equatorially after the proper

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Explanation of Plate Figures

Fig. 1. Dividing *Chaetopterus* eggs just before the pressure was applied.
Fig. 2. Forty-five seconds after the application of 330 atmospheres pressure.
Fig. 3. After 2 minutes under pressure.
Fig. 4. After 4 minutes under pressure following which the pressure was released.
Fig. 5. After 3 minutes following the release of pressure.
Fig. 6. Twelve minutes later, the cleavage being about 5–6 minutes delayed.
Fig. 7. Three minutes later.
Fig. 8. One minute later.
Fig. 9. Thirteen minutes later.
Fig. 10. Three minutes later, the cleavage being about 6–8 minutes late.
Fig. 11. Four minutes later.
Fig. 12. Two minutes later.

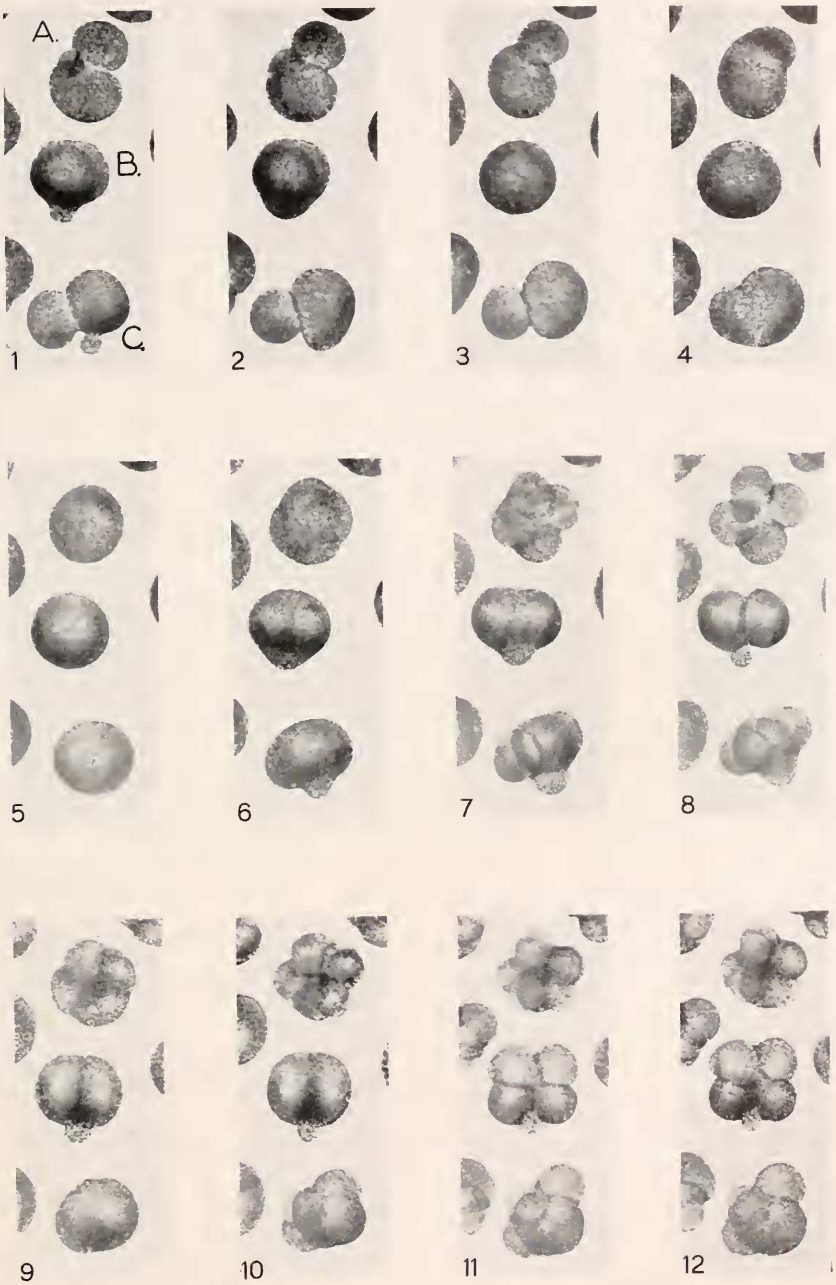

Plate I

time interval. In these respects the *Chaetopterus* egg follows a similar sequence found by Marsland (1936) in the *Arbacia* egg.

When the first cleavage is blocked in this fashion (see Figs. 1–5 in the series of photographs) and the polar lobe re-forms at the time of the second cleavage (Figs. 6–8) it has the superficial characteristics of its *first* appearance during the first cleavage of the egg. Occasionally it is abnormally small or apparently lacking, but most commonly it is as large as the first lobe, or is abnormally large as in egg *C*, Figs. 7 and 8. It frequently has a tendency to pinch off as the cleavage furrows impinge upon it, and it may not be withdrawn into the *D* blastomere during or after cleavage. It may persist as a discrete body into the 8-cell stage as in egg *B* and the probability, under those circumstances, is that it has completely pinched off. Egg *A*, Fig. 8, shows a lobe that has persisted for an abnormally long time, but which was eventually withdrawn before the third cleavage.

As a result of blocking the first cleavage, the following cleavages are very frequently abnormal. A very common abnormality is a tendency towards equal cleavage shown by egg *A* in the series of photographs. Most frequently this is brought about by the pinching off of the polar lobe as the cleavage furrows of the first and second divisions impinge upon it simultaneously, but in other more rare cases the polar lobe material is apparently equally included in all four blastomeres.

In the same sense that the cleavage was sometimes abnormally equal, it was also found with widely different unequal abnormalities. The *AB* cell has exceptionally been seen larger than the *CD* cell. The first cleavage might be normally unequal, but the second might be abnormal, producing two equal large and two equal small cells, or the *A* and *B* blastomeres might be of different size, or the *C* cell abnormally large or small in relation to the *D* blastomere.

It is clear that the time relationships between nuclear and cytoplasmic division may be somewhat disturbed by pressure treatment because, instead of four cells being produced after the normal interval for the second cleavage, not uncommonly two cells are produced (egg *B*, Fig. 8), and in certain lots of eggs the majority divided into three cells (egg *C*, Fig. 8). Frequently the blastomeres of this latter type were approximately equal in size, and a study of the photographic series demonstrated that it was generally the *CD* cell which had undergone its second cleavage equally, while the *AB* blastomere remained undivided until the next division when 6-celled eggs were produced (Figs. 11 and 12, *C*).

The most interesting group of variations were those in which the first or second cleavage furrows were equatorial. A large percentage of this type occurred in an experiment in which the second cleavage was

blocked by a pressure of 230 atmospheres applied for five minutes. At the time for the third cleavage many of these eggs were divided only by a second furrow in the equatorial plane. This phenomenon has also been exceptionally noted in eggs which were blocked only at the time of the first cleavage, and which divided only into two cells at the time of the second cleavage. This history is shown by egg *B* in the series of photographs reproduced.

In another experiment the eggs were completely blocked at the time of both the first and second cleavages by a pressure of 400 atmospheres applied for four minutes. The eggs had a tendency to become amoeboid, but the first completed cleavage of many of these eggs was equatorial and very much delayed. There was some evidence of partial furrows appearing and disappearing first in other regions of these eggs. There was, thus, considerable evidence that these eggs were multinucleate and several spindles could, in fact, sometimes be seen. The probable interpretation of all the cases in which the first or second cleavage furrow is equatorial is that these eggs have undergone two or one nuclear divisions without cytoplasmic cleavage. No cytological studies have been made and the exact relationships remain to be determined. Significantly, equatorial cleavage has never been observed before the time for the normal third cleavage.

It proved impossible to study these cleavage abnormalities in the later or trochophore stages. A negligible number of eggs subjected to pressure even became top swimmers. Complete degeneration occurred during the gastrula stage when the cells seemed to lack organization and proliferated extremely abnormally.

Conclusions

The withdrawal of the polar lobe and the rounding up of the cell under hydrostatic pressure is to be interpreted as due to the liquefaction or solation of the cortical gel which gives the lobe its structure. This is in accordance with the theory originally proposed by Marsland (1936, 1938 and 1939*a*). When the liquefaction has reduced the viscosity sufficiently surface tension forces round up the cell. The phenomenon is partly reversible and the polar lobe and cleavage furrows re-form for the following cleavages which, however, are characteristically abnormal.

Just as the cleavage furrow is able to form after centrifuging when the normal cytoplasmic constituents no longer underlie the cortex, so there is similar evidence leading to the belief that the underlying cytoplasmic elements play, at the most, a minor rôle in the formation of the polar lobe. Lillie (1906) first found that centrifuging did not hinder

lobe formation in *Chaetopterus* and expressed the view that the phenomenon was essentially "ectoplasmic." Morgan (1935) was able to hold the *Ilyanassa* egg in the centrifuge in such a way that the animal pole lay at the centrifugal end, and the centripetal oil came to lie in the polar lobe region. Such eggs produced the lobe quite normally. As an extension of this, in connection with some other unpublished work, I have ultracentrifuged *Chaetopterus* eggs with forces of 120,000 and 170,000 gravities while they were rigidly held at random and supported in a gelatin gel. When removed from the gel and fertilized these eggs cleaved normally and examples could be found with the cleavage bearing any possible relation to the axis of stratification, and even the solidly packed oil layer might be included in the lobe.

That the mitotic apparatus is not an immediate essential for polar lobe formation was first demonstrated by Wilson (1904), who showed that alternate periods of form changes occurred in the isolated lobe of *Dentalium,* and this surface activity corresponded with the succeeding cleavages of the egg. Morgan (1933 and 1935) has made a much more extensive study of these form changes in the isolated *Ilyanassa* lobe, and he has emphasized that it is presumably a cortical effect. Wilson (1929 and 1930) divided the *Chaetopterus* egg with centrifugal force and found that only the fragments derived from the vegetative hemisphere produced polar lobes. These were quite normal in spite of the loss of cytoplasmic elements. Whitaker and Morgan (1930) demonstrated the normal formation of the polar lobe in vegetative fragments of the *Chaetopterus* egg cut with a micro-needle.

The close correlation of the lobe formation with the cytoplasmic cleavage process is further demonstrated by the work of Pasteels (1934). He found that eggs taken from the Mediterranean *Chaetopterus* very late in the season often do not cleave normally. Mitotic divisions are completed but the cleavage furrows only start to form and then reverse before completion. In these eggs the polar lobe also starts to form and then is withdrawn at the proper time intervals.

As a result of the hydrostatic pressure, it is also of interest that in the following cleavages the re-formation of the lobe shows irregularities that are probably to be homologized with the irregularities of the cleavages. This is particularly true apropos of the size of the re-formed lobe which may be quite variable. It is most frequently as large as the lobe normally formed for the first division, it commonly is larger, and infrequently smaller.

Marsland (1936, 1938, and 1939), working with the equally cleaving *Arbacia* egg, had unsatisfactory material for seeing irregularities in the early cleavage pattern. As a result of this study it becomes clear that

the effects of pressure are not entirely reversible as far as the cell as a whole or the normal cleavage forces are concerned. Actually many of the abnormalities of cleavage resemble in many respects those obtained by Pasteels (1934) following ultraviolet radiation upon *Myzostoma* and *Aplysia* eggs.

Summary

1. The polar lobe of the *Chaetopterus* egg is withdrawn and the cell rounds up under a hydrostatic pressure of 220 atmospheres if the pressure is applied in the early stages of lobe formation. A pressure of 270 atmospheres is necessary if the cleavage furrow is deeply cut.

2. This phenomenon is interpreted as due to the liquefaction of the cortical gel which gives the polar lobe its structure. Further evidence is added which leads to the belief that polar lobe formation is primarily a cortical effect of the vegetative pole region, to a very large degree independent of the underlying cytoplasm or the mitotic apparatus.

3. The suppression of the polar lobe and the cleavage furrow by pressure is partly reversible in that they re-form for the succeeding cleavages, but pronounced abnormalities in the cleavage pattern are characteristic. Typical irregularities are considered, including examples in which the first or second cleavage furrows have been produced equatorially.

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